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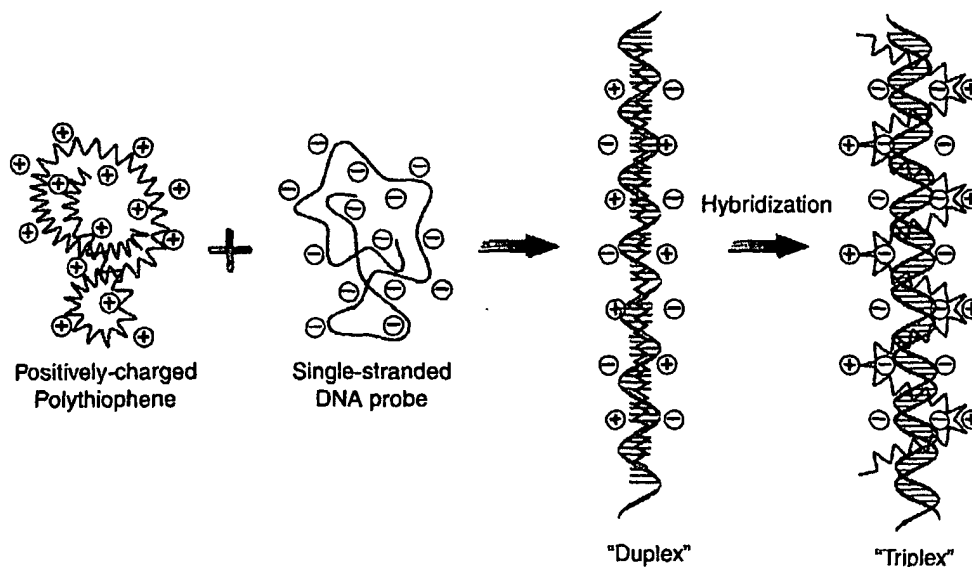
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(54) Title: DETECTION OF NEGATIVELY CHARGED POLYMERS USING WATER-SOLUBLE, CATIONIC, POLYTHIOPHENE DERIVATIVES



(57) Abstract: Novel methods allowing for the simple optical and electrochemical detection of double-stranded oligonucleotides are disclosed. The methods are rapid, selective and versatile. Advantageously, they do not require any chemical reaction on the probes or on the analytes since they are based on different electrostatic interactions between cationic poly (3-alkoxy-4-methylthiophene) derivatives and single-stranded or double-stranded (hybridized) oligonucleotides.



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**TITLE OF THE INVENTION**

DETECTION OF NEGATIVELY CHARGED POLYMERS USING WATER-SOLUBLE, CATIONIC, POLYTHIOPHENE DERIVATIVES

**5    FIELD OF THE INVENTION**

The present invention relates to simple and reliable methods for negatively charged polymer detection, namely for sequence-selective nucleic acid detection. More specifically, the present invention relates to sequence-selective nucleic acid detection methods, which are  
10    essential for the rapid diagnosis of infections and a variety of diseases.

**BACKGROUND OF THE INVENTION**

Complexes of polythiophene derivatives bearing sulfonic acid moieties and one or several adequately designed amine-containing  
15    molecules (electrostatic interactions) have been shown to be responsive to external stimuli (PCT/CA98/01082). More specifically, they were shown to undergo striking conformational changes when exposed to heat, light or various chemical and biochemical moieties giving rise to thermochromism, photochromism, ionochromism or even biochromism. These sulfonic acid-  
20    bearing polythiophene derivatives are not positively charged and thus do not have any particular affinity for negatively charged polymers.

The search for methods for sequence-selective nucleic acid detection has evolved into an important research field and has subsequently drawn the attention of researchers from various disciplines  
25    such as chemistry, physics, biochemistry, etc. As a result, some interesting DNA hybridization sensors have recently been proposed.<sup>1-5</sup>

However, most of these newly developed approaches perform detection by attaching a fluorescent or electro-active tag to the analyte.

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Assays that do not require nucleic acid functionalization prior to detection are of greater fidelity and several research groups have reported the utilization of conjugated field-responsive polymers (polypyrroles, polythiophenes, etc.) as electrochemical or optical transducers.<sup>6,7,23,24</sup> Indeed, the ability of some oligonucleotide-functionalized conjugated polymers to transduce hybridization events into an electrical or optical signal, without utilizing any labeling of the analyte, has been demonstrated.<sup>8-10</sup> The detection mechanism is based on a modification of the electrical and/or optical properties through the capture of the complementary oligonucleotides.

There thus remains a need for simpler, more sensitive and more reliable methods for the rapid and specific identification of nucleic acids. These nucleic acids could be used for the diagnosis of infections and disease. Ideally, an assay that does not require nucleic acid functionalization (chemical manipulation of nucleic acids) prior to detection nor complex reaction mixtures would have the following characteristics: it would be simpler to use than the assays currently available and it would have a high degree of fidelity. Such an assay would be highly beneficial and therefore very desirable.

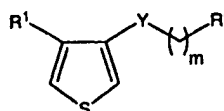
The present invention seeks to meet these and other needs.

#### **SUMMARY OF THE INVENTION**

In general terms, the present invention relates to novel cationic, water-soluble polythiophene derivatives, which can readily transduce oligonucleotide hybridization into a clearly interpretable optical (colorimetric, fluorescent or luminescent) or electrical signal. These polymers can discriminate between specific and non-specific hybridization of nucleic acids differing by only a single nucleotide.

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Specifically, the present invention relates to the synthesis and use of cationic water-soluble polymers composed of thiophene monomers having the following general formula:



- 5 wherein "m" is an integer ranging from 2 to 3; R\* is a quaternary ammonium; Y is an oxygen atom or a methylene; and R¹ is a methyl group or a hydrogen atom.

The present invention further comprises a method for detecting the presence of negatively charged polymers, comprising the steps of:

- a) contacting a complementary target to the negatively-charged polymer with a thiophene polymer to form a duplex;
- b) contacting the duplex with the negatively charged polymer; and
- c) detecting a change in electronic charge, fluorescence or color as an indication of the presence of the negatively charged polymer.

The negatively charged polymers for the method as described above, may be selected from the group consisting of: acidic proteins, glycosaminoglycans, hyaluronans, heparin, chromatographic substrates, culture substrates and nucleic acids.

20 The present invention additionally comprises a method for discriminating a first nucleic acid from a second nucleic acid, the second nucleic acid differing from the first nucleic acid by at least one nucleotide, comprising the steps of:

- a) contacting a complementary target to the first nucleic acid with a thiophene polymer to form a duplex;

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- b) contacting the duplex with the first nucleic acid, resulting in specific hybridization;
- c) contacting the duplex with the second nucleic acid, resulting in non-specific hybridization; and
- 5 d) detecting a change in electronic charge, fluorescence or color

Finally, the present invention contemplates a number of specific applications, such as the use of a positively charged polymer comprising a repeating thiophene moiety for detecting the presence of negatively charged polymers, and for purifying negatively charged  
10 polymers.

Further scope and applicability will become apparent from the detailed description given hereinafter. It should be understood however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since  
15 various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the UV-visible absorption spectrum of a  
20  $2.4 \times 10^{-5}$  M (on a monomeric unit basis) solution of: a) polymer 2; b) polymer 2 / X1 duplex; c) polymer 2 / X1 / Y1 triplex, at 55°C in 0.1M NaCl/H<sub>2</sub>O using an optical path length of 1 cm.

Figure 2 shows the UV-visible absorption spectrum of a  
 $2.4 \times 10^{-5}$  M (on a monomeric unit basis) solution of: a) polymer 2; b)  
25 polymer 2 / X1 duplex; c) polymer 2 / X1 / X1 mixture, at 55°C in 0.1M NaCl/H<sub>2</sub>O.

Figure 3 shows the UV-visible absorption spectrum of a  
 $2.4 \times 10^{-5}$  M (on a monomeric unit basis) solution of: a) polymer 2; b)

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polymer 2 / X1 duplex; c) polymer 2 / X1 / Y2 mixture, at 55°C in 0.1M NaCl/H<sub>2</sub>O.

Figure 4 shows the specific optical detection of *Candida albicans* versus *Candida dubliniensis* amplicons differing by only 2  
5 nucleotides.

Figure 5 shows a photograph of a polymer 2 – oligonucleotide duplex (solution in the left tube was colored purple-red); a polymer 2 –hybridized oligonucleotides triplex (solution in the middle tube was colored yellow); and a polymer 2 with partially hybridized  
10 oligonucleotides with two mismatches (solution in the right tube was colored pink).

Figure 6 shows the formation of a duplex and a triplex between polymer 2 and oligonucleotides.

Figure 7 shows the fluorescence intensity during specific  
15 hybridization.

Figure 8 shows the fluorescence intensity during non hybridization.

Figure 9 shows the different steps involved in the electrochemical detection of DNA hybridization.

Figure 10 shows the Cyclic voltammogram of polymer 1 onto an ITO modified electrode ( $S = 50 \text{ mm}^2$ ) in the case of perfect hybridization (straight line) and in the case of a control blank (dashed line) in 0.1 M NaCl/H<sub>2</sub>O. Scan rate 100 mV/s (two successive cycles).  
20

Figure 11 shows the Cyclic voltammogram of polymer 2 onto an ITO modified electrode ( $S = 50 \text{ mm}^2$ ) in the case of perfect hybridization (straight line) and in the case of a control blank (dashed line) in 0.1 M NaCl/H<sub>2</sub>O. Scan rate 100 mV/s (two successive cycles).  
25

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Figure 12 shows the covalent attachment of a DNA probe onto a conducting surface, its subsequent hybridization with a complementary DNA strand and its revelation with a conducting polymer.

Figure 13 shows the UV-visible absorption spectrum of a  
5  $2.4 \times 10^{-5}$  M (on a monomeric unit basis) solution of a) polymer 2, b) polymer 2 / X1 duplex, c) polymer 2 / X1 / Y1 triplex, d) polymer 2 / X1 / Y2 mixture, and e) polymer 2 / X1 / Y3 mixture, at 55 °C, in 0.1 M NaCl/H<sub>2</sub>O. Capture probe X1: 5' CATGATTGAACCATCCACCA 3' and its perfect complementary target Y1: 3' GTACTAACTTGGTAGGTGGT 5' are a DNA  
10 oligonucleotide pair specific for *Candida albicans*; capture probe X2: 5' CATGATTGAAGCTTCCACCA 3' and its perfect complementary target Y2: 3' GTACTAACTTCGAAGGTGGT 5' are a DNA oligonucleotide pair specific for *Candida dubliniensis*; Y3: 3' GTACTAACTTCGTAGGTGGT 5' is a complementary target DNA oligonucleotide designed to have one  
15 mismatch with both the *C. albicans* and the *C. dubliniensis* capture probes.

Figure 14 shows a cyclic voltammogram of polymer 2 (SH-carbon<sub>24</sub>-DNA with polymer 2) bound to an ITO modified electrode (50 mm<sup>2</sup>) in the case of perfect hybridization (X1/Y1; full line) and in the case of two control blanks (X1/X1, dotted line and X1, dashed line) in 0.1 M  
20 NaCl/H<sub>2</sub>O using a scan rate of 100 mV/s.

Figure 15 shows a cyclic voltammogram of polymer 2 (SH-carbon<sub>24</sub>-DNA with polymer 2) bound to a gold modified electrode (50 mm<sup>2</sup>) in the case of perfect hybridization (X1/Y1; full line) and in the case of a control blank (X1, dashed line) in 0.1 M NaCl/H<sub>2</sub>O. The electrodes  
25 were submitted to a -0.4 V potential for 10 minutes before scanning; the scan rate used was 50 mV/s.

Figure 16 shows a cyclic voltammogram of polymer 3 (SH-carbon<sub>24</sub>-DNA with polymer 3) bound to an ITO modified electrode (50 mm<sup>2</sup>) in the case of perfect hybridization (X1/Y1; full line) and in the case



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of two control blanks (X1, dashed line and silanized ITO (no DNA), dash-dotted line) in 0.1 M NaCl/H<sub>2</sub>O using a scan rate of 50 mV/s.

Figure 17 shows the UV-visible absorption spectrum of a  $2.4 \times 10^{-5}$  M (on a monomeric unit basis) solution of: a) polymer 1 / X1 duplex; b) polymer 1 / X1 / Y1 triplex, at 55°C in 0.1M NaCl/H<sub>2</sub>O.

Figure 18 shows the UV-visible absorption spectrum of a  $2.4 \times 10^{-5}$  (on a monomeric unit basis) solution of: a) polymer 4; b) X1 / Y1 / polymer 4 triplex; c) X1 / X1 / polymer 4 mixture, at 25°C in 0.1M NaCl/H<sub>2</sub>O.

Figure 19 shows the circular dichroism of a  $1.2 \times 10^{-4}$  M (on a monomeric unit basis) solution of polymer 3 / X1 / Y1 triplex at 55°C in 10 mM Tris buffered solution containing 0.1M NaCl.

Figure 20 shows the UV-visible spectroscopy spectrum of  $7.9 \times 10^{-5}$  M solutions in 0.1M NaCl and TE, at 55°C of a) polymer 2; b) duplex (X1 + polymer 2); c) triplex (X1 + Y1 + polymer 2); d) triplex with 2 mismatches (X1 + 5' TGGTGGATGCATCAATCATG 3'), triplex with 3 mismatches (X1 + 5' TGGTGGATACATCAATCATG 3'), triplex with 5 mismatches (X1 + 5' TGGTGGAAACAACAATCATG 3'); e) triplex with 1 mismatch (X1 + 5' TGGTGGATGCTTCAATCATG 3').

Figure 21 shows the UV-visible spectroscopy spectrum of  $7.9 \times 10^{-5}$  M solutions in 0.1M NaCl and TE, at 55°C of a) polymer 2; b) duplex (X1 + polymer 2); c) triplex (X1 + Y1 + polymer 2); d) triplex with 2 mismatches (X1 + Y2), (X1 + 5' TGGTAGATGCTTCAATCATG 3'), (X1 + 5' TGGTGGTTGCTTCAATCATG 3'), (X1 + 5' TGGTGGATGCTTTAATCATG 3'), (X1 + 5' TGGTGGATGCTTCATTCATG 3'), (X1 + 5' TGGTGGATGCTTCAATTATG 3').

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### **DETAILED DESCRIPTION OF THE INVENTION**

Other objects and attendant features of the present invention will become readily appreciated, as the same becomes better understood by reference to the following detailed description of the invention described for the purpose of illustration.

In a broad sense, the invention provides novel cationic, water soluble polythiophene derivatives that produce a clearly interpretable optical (colorimetric, fluorescent or luminescent) or electrical signal, when bound to negatively charged polymers.

The present invention provides for polymers capable of discriminating between specific and non-specific hybridization of nucleic acids differing by only a single nucleotide.

The present invention provides improved research tools. More specifically, a means for detecting nucleic acids from eucaryotic organisms as well as prokaryotic organisms such as Bacteria and Archaea.

The present invention also provides for the development of new nucleic acid detection technology, and more specifically, new detection devices based on the use of the polythiophene derivatives of the present invention.

The present invention further provides for improved clinical diagnostics, that is, the detection of infectious agents, the diagnosis of genetic diseases and tools useful for use in the pharmacogenomics field.

The present invention further provides for improved medico-legal (forensic) diagnostics, more specifically the filiation of people and animals, "forensic" tools and other genetic testing tools.

The present invention also provides for improved plant identification.

The present invention also provides for environmental and industrial screening, more specifically for the detection of genetically

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modified organisms, the detection of pathogenic agents, alimentary tracability, the identification of organisms of industrial interest (e.q. alimentary, pharmaceutical or chemical fermentation and soil decontamination).

5                   The present invention also provides for polythiophenes having an affinity for negatively charged polymers such as nucleic acids and glycosaminoglycans of natural or synthetic origin, allowing for the purification of these polymers. For example, when a polythiophene is coupled to a solid support, nucleic acids can be purified by affinity and / or  
10   ion exchange chromatography.

                  The present invention also provides for polythiophenes that are thermostable and autoclavable, allowing for a wide range of applications.

                  The present invention further provides methods and tools  
15   by which negatively charged polymers such as acidic proteins (kinesins), glycosaminoglycans (hyaluronans, heparin) and any natural or synthetic negative polymers can be detected or blocked by binding with the polythiophenes.

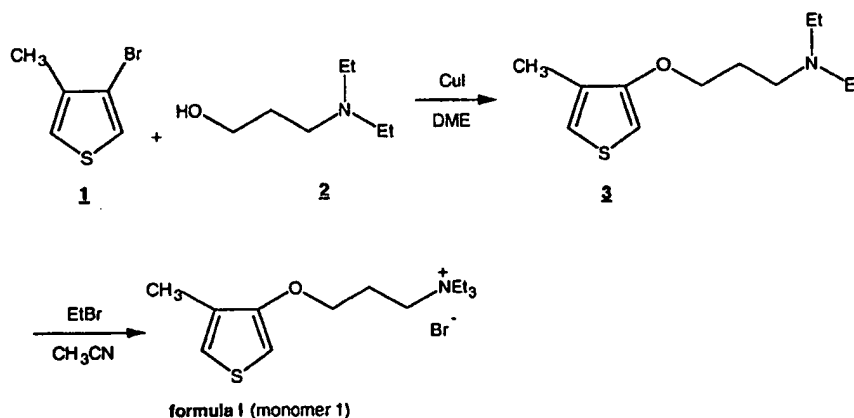
                  Advantageously, this novel approach is rapid, specific,  
20   sensitive, and highly versatile, yet simple. It is based on the different electrostatic interactions and conformational structural changes between single-stranded or double-stranded negatively-charged oligonucleotides or nucleic acid fragments, and cationic electroactive and photoactive poly(3-alkoxy-4-methylthiophene) derivatives. It allows a single reagent assay  
25   procedure for genomic analysis and molecular diagnostics. Furthermore, the above mentioned detection methods can also be used for solutions of nucleic acids, nucleic acids separated by gel electrophoreses, nucleic acids fixed onto solid supports such as glass slides or plates, silicon chips or other polymers.

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## Synthesis

Set forth below are preferred synthesis schemes for the preparation of the water-soluble, cationic, electroactive and photoactive poly(3-alkoxy-4-methylthiophene)s.

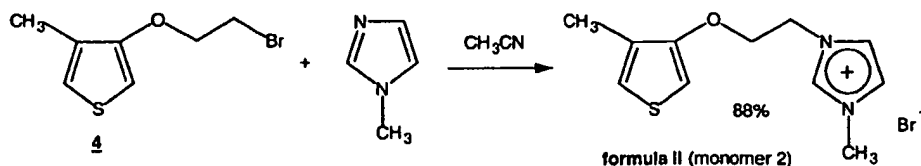
5                   The synthesis of monomer 1 is carried out in a two-step  
procedure, starting from 3-bromo-4-methylthiophene (Aldrich Co.) (see  
Scheme 1). The first step is a nucleophilic substitution reaction onto the  
thiophene ring catalyzed by CuI, as reported by El Kassmi et al.<sup>11</sup> The  
second step involves a quaternization reaction between the tertiary amine  
10 and 1-bromoethane in acetonitrile.<sup>12</sup>



### Scheme 1

Similarly, monomer 2 is prepared from 3-(2-bromoethoxy)-4-methylthiophene (compound 4) and 1-methylimidazole (Aldrich Co.) (see Scheme 2). Compound 4 is prepared according to the procedure developed by Leclerc et al.<sup>13</sup> The quaternization reaction between 1-methylimidazole and compound 4, provides the desired monomer imidazolium salt 2.<sup>14</sup>

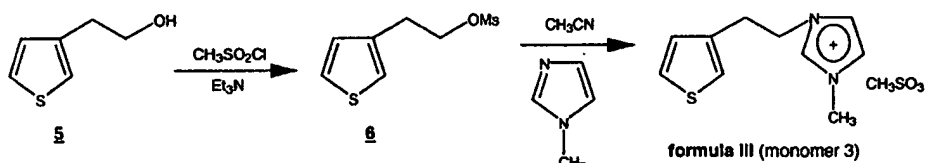
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Scheme 2

Similarly, the synthesis of monomer 3 involves the conversion of 3-thiopheneethanol (compound 5) into its corresponding mesylate protected derivative (compound 6). The quaternization reaction

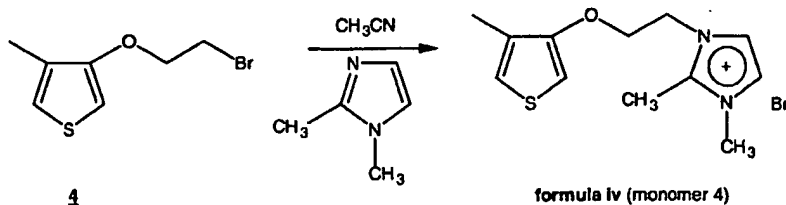
10 between 1-methylimidazole and compound 6, provides the desired monomer imidazolium salt 3.



Scheme 3

The synthesis of monomer imidazolium salt 4 involves a

15 quaternization reaction of compound 4 with 1,2-dimethylimidazole.

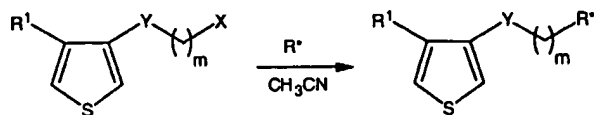


Scheme 4

A more general procedure reflecting the preparation of cationic thiophene monomers is depicted in scheme 5, wherein "m" is an integer equal to 2 or 3; "Y" is an oxygen atom or a methylene group; and

20 R<sup>1</sup> is a hydrogen atom or a methyl group.

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Scheme 5

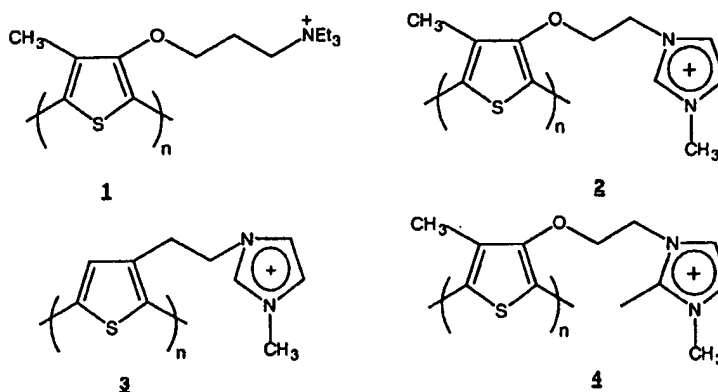
When  $R^*$  is  $\text{Et}_3\text{N}$ ,  $Y$  is an oxygen atom and  $R^1$  is a methyl group, then “ $m$ ” is equal to 3. When  $R^*$  is 1-methylimidazole,  $Y$  is an oxygen atom and  $R^1$  is a methyl group, then “ $m$ ” is equal to 2. When  $R^*$  is 1-methylimidazole,  $Y$  is a methylene group and  $R^1$  is a hydrogen atom, then “ $m$ ” is equal to 2. When  $R^*$  is 1,2-dimethylimidazole,  $Y$  is an oxygen atom and  $R^1$  is a methyl group, then “ $m$ ” is equal to 2.

The inherent chemical and physical properties of imidazole provide for a wide electrochemical window, favorable for electrochemical detection.<sup>15</sup>

All polymers, more specifically the cationic, water soluble, electroactive polymers 1, 2, 3 and 4 (Scheme 6), were synthesized by oxidative chemical polymerization of the corresponding monomers using  $\text{FeCl}_3$  or  $\text{K}_2\text{S}_2\text{O}_8$  as the oxidizing agent in chloroform. This method of polymerization yields well-defined regio-regular 3-alkoxy-4-methylthiophene polymers (1, 2 and 4) as well as a non-regioregular 3-alkylthiophene polymer (3), having an average molecular weight of about 5 kDa and a polydispersity index of ca. 3.<sup>16</sup> Note that “ $n$ ” can vary from 3 to about 100. The resulting polymers (using  $\text{FeCl}_3$  as the oxidizing agent) contain a mixture of anions such as  $\text{FeCl}_4^-$ ,  $\text{Cl}^-$  and  $\text{Br}^-$ . In order to produce a cationic polymer with only one specific counter anion (e.g. hydrophilic counter anions like  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CH}_3\text{SO}_3^-$ , etc. or hydrophobic counter anions like  $\text{BF}_4^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{PF}_6^-$ , etc.), an anionic-exchange reaction is performed by dialysis or precipitation. As expected, all resulting polymers were found

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to be soluble in aqueous solutions when in the presence of hydrophilic anions.



Scheme 6

5

As any water-soluble cationic polyelectrolytes, the polythiophene derivatives of the present invention can make strong complexes with negatively-charged oligomers and polymers.<sup>17</sup> This complexation results in the formation of complexes having specific optical properties. For instance, at 55°C, aqueous solutions (0.1 M NaCl or 10 mM Tris buffer / 0.1M NaCl) of the cationic polymer 2 are yellow ( $\lambda_{\text{max}} = 397$  nm). This absorption maximum at a relatively short wavelength is related to a random coil conformation of the polythiophene derivative.<sup>6</sup> After the addition of one equivalent (on a monomeric unit basis) of a given oligonucleotide (20-mers), the mixture becomes red ( $\lambda_{\text{max}} = 527$  nm) due to the formation of a so-called duplex. After 5 minutes of mixing in the presence of one equivalent of a complementary oligonucleotide, the solution becomes yellow ( $\lambda_{\text{max}} = 421$  nm) presumably due to the formation of a new complex (triplex) (Figures 1 and 5).

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A schematic description of these conformational transitions for both types of polyelectrolytes is given in Figure 6.

Based on previous studies on thermochromic, solvatochromic and affinitychromic regioregular poly(3-alkoxy-4-methylthiophene)s,<sup>6</sup> it is believed that these colorimetric effects are made possible due to the different conformational structure of the conjugated polymer in the duplex (highly conjugated, planar conformation) compared to that observed in the triplex (less conjugated, non-planar conformation) and to a stronger affinity of the conjugated polymer for the double-stranded oligonucleotides (nucleic acids) ( $1 \times 10^5 \text{ M}^{-1}$ ) than that measured for single stranded oligonucleotides (nucleic acids) ( $5 \times 10^4 \text{ M}^{-1}$ ).

In a control experiment it was demonstrated that the addition to the solution of an oligonucleotide identical to that of the capture probe results in no color change (Figure 2).

In order to verify the specificity of these complexations, two pairs of complementary oligonucleotides (20-mers) differing by only 1 or 2 nucleotides were synthesized (Table 1) and carefully investigated. A slight but distinct change in the UV-visible absorption spectrum is observed in the case of the oligonucleotide target having 2 mismatches. Even with only one mismatch, it is possible to distinguish between a perfect and a non-perfect hybridization (Figure 13). In this case, the colorimetric difference is mainly based on different kinetics of complexation, since similar yellow aqueous solutions are observed after 30-60 minutes of mixing at 55 °C. However, it is possible to stop the hybridization reaction after 5 minutes of mixing, by placing the solutions at room temperature. Following these procedures, stable yellow and orange solutions are obtained (curves c and e) (Figure 13). The detection limit of this colorimetric method is about  $1 \times 10^{13}$  molecules of oligonucleotide (20-mers) in a total volume of 100  $\mu\text{L}$ .



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Very similar results have been obtained for polymer 1 (Figure 17) and polymer 4 (Figure 18) and various oligonucleotides. Figure 20 shows the UV-Visible absorbance spectrum of polymer 2 when using target oligonucleotides ranging from 0 to 5 mismatches. Figure 21 illustrates the UV absorbance results of polymer 2 using the target oligonucleotide always having two mismatches at different positions. These results show that the polymer can discriminate between perfectly matched and mismatched hybrids, independently of the nature of the mismatched nucleotide bases, and independently of the position or the length of the mismatches. Moreover, it is even possible to discriminate a single mismatch from multiple mismatches.

**Table 1:** Synthetic oligonucleotides to study the specificity of hybridization.

Oligonucleotides specific for <i>Candida albicans</i>	Oligonucleotide specific for <i>Candida dubliniensis</i>
<b>X1</b> 5' CATGATTGAACCATCCACCA 3'	<b>X2</b> 5' CATGATTGAAGCTTCCACCA 3'
<b>Y1</b> 3' GTACTAACTTGGTAGGTGGT 5'	<b>Y2</b> 3' GTACTAACTTCGAAGGTGGT 5'
<b>Y3 (designed to have one mismatch wit X1 and X2)</b> 3' GTACTAACTTCGTAGGTGGT 5'	

The concentration of a particular sequence region of DNA can be amplified by using a polymerase chain reaction (PCR), and the present colorimetric method can be extended to these PCR products. Indeed, the introduction of the polymerase chain reaction (PCR) has solved

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the problem of detecting small amounts of DNA and the polymers of the present invention can be used in the identification of PCR products. UV spectroscopic results in the absorbance range of 430-530 nm (Figure 4) have illustrated the specific optical detection of *Candida albicans* and  
5 *Candida dubliniensis* amplicons, which differ by only 2 nucleotides, by a polymer 2 / X1 duplex. Such detection was carried out in 45 minutes directly from a PCR product at a concentration normally generated in a 100 $\mu$ L PCR volume (ca.  $3 \times 10^{12}$  copies). Experimental details on PCR and choice of target sequences for identification of *Candida* are provided in a  
10 co-pending patent application (PCT/CA00/01150).

In addition, as shown in Figure 19, circular dichroism (CD) measurements reveal an optical activity for polymer 3 in its random coil, a bisignate CD spectrum centered at 420 nm in the triplex, characteristic of a right-handed helical orientation of the polythiophene  
15 backbone. Such a right-handed helical structure is compatible with binding of the polymer to the negatively-charged phosphate backbone of DNA. The thermal stability study by UV or CD measurements shows a different thermal stability between a duplex and a triplex, and this property could be extremely useful for more stringent washing conditions.

20 A fluorometric detection of oligonucleotide hybridization is also possible based on the difference in the fluorescence quantum yield of the positively-charged poly(3-alkoxy-4-methylthiophene) in the random coil (the isolated state) or in the aggregated state.<sup>16</sup> For instance, at 55 °C, the yellow appearance of polymer 2 is fluorescent (quantum yield of 0.03) but  
25 upon addition of one equivalent of a negatively-charged oligonucleotide, the intensity of the emission spectrum is strongly decreased (quenched). In the case of perfect hybridization, the polymeric triplex gives a stronger emission (Figure 7). Upon addition of the same oligonucleotide, no hybridization occurs and the solution does not show any change in

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fluorescent intensity (Figure 8). Using a laser as the excitation source, very low limits of detection are obtained. It is possible to detect the presence of as few as  $3 \times 10^6$  molecules of the complementary oligonucleotide (20-mers) in a volume of 200  $\mu\text{L}$ , which corresponds to a concentration of  $2 \times 10^{-14}$  M. Moreover, by covalently attaching the oligonucleotide to a fluorescent-conjugated polymer, or by using an optimized fluorescence detection scheme based on a high intensity blue diode (excitation source) and a non-dispersive, interference filter-based system, an even more sensitive and more specific detection capability is achieved.

10                   The electrochemical properties of polymers 1 and 2 can be used for the detection of DNA hybridization in aqueous solutions, as shown in Figure 9.<sup>8,10</sup> Using the layer by layer deposition techniques,<sup>25,26</sup> the first step required the binding of a capture probe composed of single-stranded oligonucleotide X1 to an ammonium-functionalized indium tin oxide (ITO) surface.<sup>18,27</sup> After rinsing with pure water, the modified electrode so-obtained was dipped and hybridized in the presence of a complementary oligonucleotide Y1. The resulting electrode was revealed with an aqueous solution of positively-charged polymer 1 or 2 ( $10^{-4}$  M on a monomeric basis), which provides a signal that is a function of the amount of DNA present on the surface. As a control experiment, an aqueous solution of oligonucleotide X1 was added to the X1 modified ITO electrode, and then transferred into an aqueous solution of polymer 1 or 2. In this way, these polymers serve as "mass transducers" for the oligonucleotide present in the sample.

25                   The detection of DNA hybridization is further demonstrated by the following two examples using different cationic poly(3-alkoxy-4-methylthiophene)s (polymers 1 and 2). The so-obtained results are illustrated in Figures 10 and 11.

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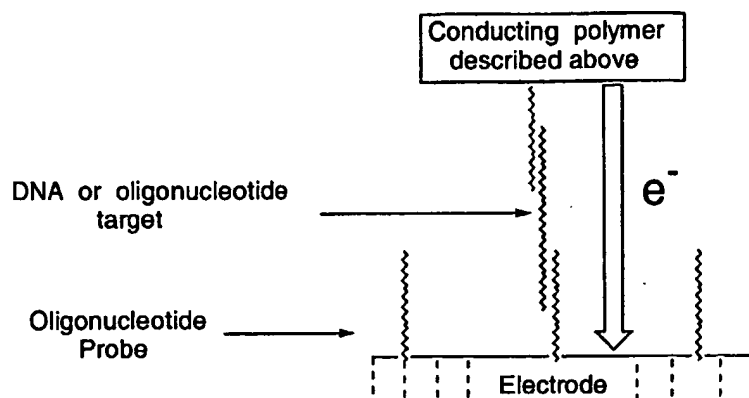
In both cases, the maximum anodic current is more important in the case of perfect hybridization as compared to the blank control. In addition, a shift to a higher potential (ca. 40-50 mV) is observed when specific hybridization occurs (38 mV for polymer 4 as compared to 52 mV for polymer 2). The higher oxidation current can be explained by the stronger affinity of the polymers for double-stranded oligonucleotides, whereas the positive shift of oxidation potential is explained by the formation of a less conjugated structure in the case of specific hybridization. This is in agreement with previous optical measurements.

10                   An assay using a smaller electrode [ $S(\text{surface}) = 10 \text{ mm}^2$ ] has allowed the detection of  $2 \times 10^{11}$  molecules of oligonucleotide (20-mers). This very simple electrochemical methodology is already more sensitive, by two orders of magnitude, than the best results obtained with electrochemical methods using oligonucleotide-functionalized conjugate polymers.<sup>10</sup> Clearly, by decreasing the size of the electrodes and by  
15                   increasing the size of the target molecules, much lower detection limits should be obtained.

                  In order to further enhance the specificity of the detection, the oligonucleotide probe can be covalently attached to the polythiophene derivatives  
20                   

                  The detection of DNA sequences can also be carried out electrochemically as shown below in Scheme 7.

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Scheme 7

The DNA probes can be covalently fixed to a conductive surface (Figure 12). This allows for the linking of a larger number of DNA probes to the surface, improving the specificity and detection limit for a small surface.

The first step involves the modification of the conductive substrate (ITO, SnO<sub>2</sub>, gold, doped silicon or other conductive substrate) by the covalent attachment of a single-stranded DNA probe. The complementary DNA strand is hybridized and the polymer is captured on the hybridized probe. The observed electrochemical signal is different for a hybridized probe (ds-DNA) versus a non-hybridized probe (ss-DNA). A linker can be used to attach the DNA probe to the conductive surface. The end groups of the linker are such that one end readily reacts with the conductive substrate to form a covalent bond, whereas the other end readily reacts with an "end-modified" (SH, NH<sub>2</sub>, COOH, etc.) DNA probe, with or without a spacer (carbon<sub>24</sub>) between the reactive function and the DNA.<sup>19,20,21</sup> The end group of the linker that reacts with the conductive substrate's surface can be a silane derivative, such as an alkoxy silane, or a chlorosilane. The other end group of the linker can be composed of an

- 20 -

aldehyde, a carboxylic acid, a primary amine, a succinimide ester moiety or other functional groups capable of reacting with "end-modified" DNA probes, that is, DNA probes having specific end groups, resulting in the formation of covalent bonds with or without the help of coupling agents.

5                   The immobilization of an oligonucleotide by means of a thiol group can be carried out through its 3' or 5' terminal group. Accordingly, DNA was immobilized onto an ITO surface following the literature procedure for immobilizing DNA on a glass surface.<sup>28, 29, 30</sup>

10                   It is also possible to link "end-modified" DNA probes to various other surfaces such as gold or other metals and metal oxides.<sup>22</sup> Non-metallic surfaces such as beads, glass slides, optical fibers or any other suitable non-metallic solid support, are also possible. The immobilization of DNA onto a gold surface is accomplished following known published techniques, and the density of the attached probes can  
15                   vary depending on the concentration and reaction times.<sup>31, 32</sup> The hybridization efficiency can be optimized by heating the substrate before adding the complementary strand.<sup>31, 33</sup> Polymer deposition can be optimized by dipping the substrate vertically into a polymer solution, and by varying the salt concentration and the temperature of the polymer solution.  
20                   The washes can be further optimized to limit the contribution of the polymer with the non-hybridized probe.

                  The signal observed for a triplex should be stronger than that observed for a duplex, with possibly a small shift towards higher potentials when hybridization occurs. The amount of DNA is higher in the  
25                   case of a triplex, which implies the presence of a higher amount of negative charges. It is suspected that there might be two equivalents of polymer (positive charge) binding in the case of a triplex. Moreover, in the case where only one equivalent of polymer is bound to DNA, the polymer on a non-hybridized probe (ss-DNA) is more easily washed off, as

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compared to a polymer bound to DNA on a hybridized probe (ds-DNA), since it is less stable at high salt content or elevated temperatures. By washing off essentially all of the polymer from the duplex while leaving the triplex intact, a signal coming directly from the triplex (hybridized probe + polymer) is assured. Finally, targets having mismatches will give a different  
5 signal and possibly a lower current, due to less perfect hybridization.

The electrochemical detection of DNA hybridization is further demonstrated by using polymer 2 and polymer 3, as illustrated in Figures 14-16.

10

### Experimental section

#### EXAMPLE 1

General procedure for the aromatic nucleophilic substitution on thiophene;

15

#### Synthesis of compound 3

Sodium hydride (0.4g, 15 mmol) was added between 0 and 10°C under nitrogen to a solution of N,N-diethylpropanolamine (2.0 g; 15.2 mmol) in 50mL of DME, and the resulting mixture stirred at ambient  
20 temperature for 20 minutes. 3-Bromo-4-methyl thiophene (2.0g, 11.3 mmol) dissolved in 20 mL of DME (20 mL) and CuI (1.07g, 5.65 mmol) were then added to the reaction mixture. The mixture was subsequently stirred overnight at 95°C, while under nitrogen, diluted with methylene chloride and filtered. The organic phase was washed three times with  
25 water, dried over MgSO<sub>4</sub> and evaporated. The crude product was purified by chromatography on silica gel using CH<sub>2</sub>Cl<sub>2</sub> as the eluent, followed by the use of MeOH.

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**Compound 3:** Yield 26% ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.04 (t, 6H); 1.93 (m, 2H); 2.09 (s, 3H); 2.57 (m, 6H), 3.97 (t, 2H); 6.14 (d, 1H); 6.81 (d, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 11.69; 12.50; 26.84; 46.91; 49.26; 68.19; 95.88; 119.57; 129.05; 156.04; MS m/e: Calcd. For  $\text{C}_{12}\text{H}_{21}\text{N}_1\text{O}_1\text{S}_1$ ; 227.1344;

5 Found: 227.1347.

### EXAMPLE 2

General procedure for quaternization reaction: Synthesis of monomer 1.

10 1-Bromoethane (6 mL, 80.4 mmol) was added to a solution of compound 3 (0.4g, 1.8 mmol) in 60 mL of acetonitrile. The reaction mixture was stirred at 70°C under nitrogen for 3 days. After evaporation of the acetonitrile, the crude product was crystallized from ethyl acetate as a colorless powder.

15 **Monomer 1:** Yield 100%; m.p. 145-147°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.41 (t, 9H); 2.06 (s, 3H); 2.31 (m, 2H); 3.57 (m, 8H), 4.12 (t, 2H); 6.24 (d, 1H); 6.84 (d, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.87; 12.61; 22.55; 53.61; 54.66; 65.80; 97.18; 120.28; 128.26; 154.77.

20

### EXAMPLE 3

Synthesis of monomer 2.

1-Methyl-imidazole (1.0 mL, 12.3 mmol) was added to a solution of product 4 (0.54g, 2.46 mmol) dissolved in  $\text{CH}_3\text{CN}$  (35 mL). The  
25 resulting reaction mixture was stirred at 70°C for two days. After evaporation of the solvent, the crude product was washed twice with warm ethyl acetate and twice with diethyl ether at room temperature to provide monomer 2 as a pure white solid compound.



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**Monomer 2:** Yield 88%; 92-94°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 2.05 (s, 3H); 4.09 (s, 3H); 4.38 (t, 2H); 4.90 (t, 2H), 6.27 (d, 1H); 6.82 (d, 1H); 7.63 (s, 1H); 7.68 (s, 1H); 10.24 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ: 12.81; 36.76; 49.36; 68.01; 97.92; 120.68; 123.26; 123.30; 128.35; 137.51; 154.23.

5

#### EXAMPLE 4

##### Synthesis of monomer 3

Methanesulfonyl chloride (2.4 mL; 31.2 mmol) was added dropwise to a solution of 3-thiopheneethanol (2.0 g; 15.6 mmol) and triethylamine (4.3 mL; 31.2 mmol), in dichloromethane (50 mL). The reaction mixture was stirred at room-temperature for two hours. The organic phase was washed with a NaHCO<sub>3</sub> solution, followed by several washings with water, and was finally dried with MgSO<sub>4</sub> and concentrated.

15 The crude product was purified by silica gel chromatography using CH<sub>2</sub>Cl<sub>2</sub>/hexane (1/1) as the eluent to yield compound 6 (59%); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25°C, TMS): δ= 2.87 (s, 3H); 3.08 (t, 2H); 4.40 (t, 2H); 6.98 (d, 1H); 7.09 (d, 1H); 7.29 (dd, 1H). <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>, 25°C, TMS): δ= 29.99; 37.19; 69.59; 122.24; 125.98; 127.98; 136.44.

20 1-Methyl imidazole (0.4 g; 4.85 mmol) was added to a solution of compound 6 ( 0.2 g; 0.97 mmol) in toluene (20 mL). The reaction mixture was stirred at 94 °C for two days. Following evaporation of the solvent, the crude product was washed with warm ethyl acetate to yield monomer 3 as a liquid.

25 **Monomer 3:** Yield (95%); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25°C, TMS): δ= 2.72 (s, 3H); 3.19 (t, 2H); 3.90 (s, 3H); 4.49 (t, 2H); 6.95 (d, 1H); 7.06 (d, 1H); 7.24 (m, 2H); 7.33 (s, 1H); 9.68 (s, 1H). <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>, 25°C,

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TMS):  $\delta$ = 30.77; 36.10; 39.67; 50.06; 122.24; 122.81; 123.01; 126.46; 127.66; 136.08; 137.80.

#### EXAMPLE 5

##### 5                                      Synthesis of monomer 4

The quaternization reactions were carried out following the procedure described in example 3.

**Monomer 4:** Yield 85%;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ , 25°C, TMS):  $\delta$ = 2.02  
10 (s, 3H); 2.89 (s, 3H); 3.96 (s, 3H); 4.39 (t, 2H); 4.85 (t, 2H); 6.27 (d, 1H); 6.83 (d, 1H); 7.57 (d, 1H); 7.96 (d, 1H).

#### EXAMPLE 6

General procedure for the chemical polymerization: Synthesis of polymer 1  
15

To a solution of iron trichloride (0.94g, 5.8 mmol) in chloroform (23 mL) under nitrogen, a solution of monomer 1 (0.487 g, 1.4 mmol) in chloroform (15 mL) was added dropwise. The mixture was stirred at room temperature for a period of 2 days. The reaction mixture was  
20 evaporated to dryness and the crude product washed quickly with methanol, and dissolved in excess of acetone, and precipitated by the addition of an excess of tetrabutylammonium chloride or tetrabutylammonium bromide. The black-red polymer was dissolved in methanol and dedoped by adding a few drops of hydrazine. The final  
25 solution was evaporated. The resulting polymer was washed several times with a saturated solution of tetrabutylammonium chloride or tetrabutylammonium bromide in acetone, and by Soxhlet extraction with acetone over a period of 6 hours, and then dried under reduced pressure to yield polymer 1 (0.32g, 66%).

- 25 -

### EXAMPLE 7

#### General procedure for optical detection

##### i) Synthetic oligonucleotides

5                    In a quartz UV cuvette, 100 $\mu$ L ( $7.47 \times 10^{-8}$  repeat units (RU) of positive charges) of a solution of polymer 2 were added to an aqueous solution (3 mL) containing either 0.1M NaCl or 10 Mm Tris buffer plus 0.1M NaCl (pH= 8). The mixture was heated at 55°C for 5 min and had a yellow appearance. 12 $\mu$ L ( $7.47 \times 10^{-8}$  RU of negative charges) of  
10 oligonucleotide solution (capture probe) were then added and the resulting red solution kept at 55°C for an additional 5 minutes. The appropriate oligonucleotide target was added to the solution at 55°C over 5 minutes. A final yellow color is indicative of a positive result, meaning that perfect hybridization has taken place. On the other hand, a red or a red-pink color  
15 is representative of non specific or partial hybridization (two mismatches), respectively (Figures 1-3, respectively).

##### ii) PCR products

20                    The amplicon (double stranded 149 base pairs) from the PCR product was pre-purified by «QIAquick» purchased from Qiagen. H<sub>2</sub>O (90  $\mu$ L) was added to a centrifuge tube followed by the addition of 6.7  $\mu$ L ( $5.03 \times 10^{-9}$  mol of positive charge) of a solution of polymer 2, followed by the addition of the oligonucleotide capture probe Y1 (2  $\mu$ L;  $5.03 \times 10^{-9}$  mol of negative charge) and finally, by the addition of NaCl 1M (20  $\mu$ L). The  
25 resulting mixture was heated at 50°C for 10 min. The purified PCR product was freshly denatured, cooled in ice water and then added to the above solution. The hybridization reaction was kept at 50°C for 35 min and the color change observed either visually or by UV measurement (Figure 4).

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#### EXAMPLE 8

##### General procedure for fluorescent detection

A procedure identical to that described for optical  
5 detection was employed, with the exception of the use of a  
fluorospectrometer. The fluorescent intensity of a "duplex" (association  
between a positively charged polymer and an oligonucleotide capture  
probe) was weak or insignificant (practically zero) due to the fluorescent-  
quenching property of the aggregated form of the polymer. When perfect  
10 hybridization does occur, the fluorescent signal becomes more significant  
(Figure 7).

#### EXAMPLE 9

##### General procedure for electrochemical detection

15

The electrochemical test for hybridization was performed  
in conjunction with a control blank. 60  $\mu\text{L}$  ( $1.44 \times 10^{-8}$  mol of negative  
charge) of captured oligonucleotide Y1 were deposited on the aminated  
ITO electrode ( $S = 50 \text{ mm}^2$ ) at ambient temperature for 5 min. After  
20 washing with water, 60  $\mu\text{L}$  ( $1.44 \times 10^{-9}$  mol of negative charge) of the target  
oligonucleotide X1 were added and the hybridization carried out at  $55^\circ\text{C}$   
over 20 minutes. The electrode was then cooled to room temperature over  
10 min and washed twice with 0.3 M NaCl, 0.03 M NaOAc and 0.1% SDS  
(pH 7) and water. 100  $\mu\text{L}$  ( $1 \times 10^{-8}$  mol of positive charge) of a solution of  
25 polymer 1 or 2 was spread on the modified electrode for 5 min, followed by  
washing with  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1/4) and water. Cyclic voltammograms were  
performed in aqueous 0.1 M NaCl solutions (Figures 10 and 11).

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EXAMPLE 10

General procedure for the preparation of electrodes substituted with covalent probes

5 i) Covalent attachment of DNA probes to an ITO electrode (polymer 2)

ITO slides are sonicated in hexanes (10 min), methanol (10 min) and ultrapure water (10 min), and are then treated with an aquaregia solution ( $\text{H}_2\text{O}_2/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 1/5/1) at  $40^\circ\text{C}$  over a 30 minute period. The resulting slides are rapidly washed with water, followed by  
10 sonication in water and in acetone, then dried with air, nitrogen or argon, and heated to  $110^\circ\text{C}$  for 2 to 10 min. Following this, the slides are submerged for three hours, while under an inert atmosphere, in an acidified ethanol solution (1mM acetic acid in 95% ethanol) containing 5% mercaptopropyltrimethoxysilane, followed by sonication in fresh ethanol  
15 (95%) and in ultrapure sterile water. Finally, the slides are heated at  $110^\circ\text{C}$  for at least one hour, and cooled to room temperature before modification with DNA.

The washes, following DNA deposition, hybridization and polymer deposition, are carried out using an orbital shaker, unless  
20 otherwise stated. DNA attachment is carried out using a solution of SH-carbon<sub>24</sub>-DNA in sodium citrate buffer (30 mM, pH=4, 50  $\mu\text{L}$ ) which is deposited onto each ITO slide, each spot forming a circle of about 1 cm in diameter. The deposition reaction is performed in a humidified chamber over a period of 20-24 hours. Any non-deposited DNA is drained off, and  
25 the slides washed with 5x SSC + 0.1% Tween 20, followed by washings with 1x SSC + 0.1% Tween 20 and NaCl 0.1M.

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ii) Hybridization (ITO, polymer 2)

50  $\mu$ L of RC-DNA (Y1) or of probe X1 (blank test) (25  $\mu$ M in 2xSSC), are deposited onto the DNA spot. The slides are then heated to 55°C for about 3 hours while in a humid atmosphere, and then cooled to room temperature for 15 minutes. Any non-reacted DNA is drained off and the slides washed with 2x SSC, NaCl (0.1M), with 1x SSC+0.1% Tween 20 and with NaCl (0.1M).

iii) Polymer deposition (ITO, polymer 2)

10 Polymer 2 is deposited onto the electrodes. The slides are dipped vertically over a period of 5 minutes in a solution of polymer 2 (2 mL of  $10^{-4}$  M in 0.01M NaCl) at room temperature. The slides are then dipped in 2 mL of a NaCl solution (0.01M), followed by dipping in 2mL of a  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1/4) solution and finally, by dipping in 2mL of a NaCl solution  
15 (0.01M).

iv) Hybridization (ITO, polymer 3)

30  $\mu$ L of Y1 (2.5  $\mu$ M/NaCl 0.1M) are inserted into hybridization chambers which are placed onto the surface of the ITO electrode. The slides are heated for about two hours at 55°C while in a humid atmosphere, and then cooled to room temperature. The hybridization chambers are removed and the slides washed with a 0.1M NaCl solution and finally dried with argon.

25 v) Polymer deposition (ITO, polymer 3)

Polymer 3 is deposited onto the electrodes. A 30  $\mu$ L aqueous solution of polymer 3 ( $10^{-4}$ M) is inserted into hybridization chambers which are then placed onto the surface of the ITO electrode. The slides are heated at 55°C for 20 minutes. The hybridization chambers are

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removed and the slides washed with a first portion of a 0.8M NaCl solution at 55°C, then washed with a new portion while cooling to room temperature. The slides are finally rinsed at room temperature with a 0.1M NaCl solution.

5

vi) Covalent attachment of DNA probes to a gold electrode (polymer 2)

Gold slides are rinsed sequentially with hexanes, methanol and water. The plates are then treated with a pyranah solution ( $\text{H}_2\text{O}_2$  30% /  $\text{H}_2\text{SO}_4$  concentrated; 30/70) over a period of about 15 minutes.

10 The slides are then thoroughly washed with nanopure sterile water, and dried with argon.

DNA deposition is performed under inert atmosphere using 50  $\mu\text{L}$  of a solution of SH-carbon<sub>24</sub>-DNA (25  $\mu\text{M}$  in 1M phosphate buffer (pH=7);  $\text{K}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$ ). The solution is deposited onto a 1 $\text{cm}^2$  surface, and treated for about 16 hours under inert atmosphere. Any non-  
15 reacted DNA is then drained off, and the slides sequentially washed with  $\text{H}_2\text{O}$ , 2x SSC, and sterile water.

Polymer 2 is deposited onto the electrodes. A 50  $\mu\text{L}$  solution of polymer 2 ( $10^{-5}$  M in 0.1M NaCl) is deposited onto the DNA spot  
20 and the slides heated to 55°C for about 30 minutes. The slides are then washed repeatedly with a NaCl solution (0.1M) at room temperature.

Note that the order of additions does not have to follow that described in the above example, that is, a complementary (or a non-complementary or with mismatches) DNA strand is added to a covalently  
25 linked DNA strand, followed by the addition of a polymer solution. The polymer solution may be added prior to the addition of the complementary (or a non-complementary or with mismatches) DNA strand.

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EXAMPLE 11

The use of the polymers of the present invention to purify nucleic acids and other such negatively charged molecules

5                   The polymers described in the present invention have high affinity for negatively charged molecules, especially nucleic acids. In addition, they are soluble in aqueous solution and very stable under a wide range of temperatures. Therefore, it is possible to use these properties to purify nucleic acids and other negatively charged molecules.

10   Chromatographic separation would involve the following steps: (1) immobilizing the polymers; (2) applying the analyte to be separated onto the immobilized polymers under conditions such that electrostatic interactions are possible between the analyte and the polymer; and (3) eluting the analyte by applying conditions where electrostatic interactions

15   between the analyte and the polymer are not favored.

                  Immobilization of the polymer can be achieved by covalently coupling the polymer to a suitable solid support such as glass beads, or to beads made of other types of polymers. Alternatively, coupling to a solid support could be achieved via electrostatic interactions, such as

20   that demonstrated in Example 10. In the latter case, where a capture oligonucleotide is covalently attached to a solid support, the polymer would be expected to be eluted along with the analyte in the final elution step since it is attached only by electrostatic bonds. Color changes could even be used to monitor the chromatographic process.

25                   Conditions for binding the analyte to the polymer would preferably involve solutions of low ionic charge, such as water, 0.1 M NaCl and 10mM Tris buffer/0.1 M NaCl. Different washing conditions can be applied to remove any unwanted fractions of the analyte. pH changes



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could alternatively be used to obtain conditions for binding the analyte to the polymer.

Conditions for eluting the analyte involves solutions of high ionic charge, such as 1.0 M NaCl solution or any other solution comprising a sufficiently high counter ion concentration capable of competing for the electrostatic interactions with the polymer. Again, pH changes could alternatively be used to obtain conditions for eluting the analyte.

10

#### EXAMPLE 12 Detection of messenger RNA

Purified, *in vitro* transcribed, polyadenylated messenger RNA of *Arabidopsis thaliana* gene coding for NAC1, was purchased from Stratagene. Perfectly matched complementary DNA oligonucleotide N1 (5' CGAGGCTTCCATCAATCTTA 3') was synthesized by phosphoramidite chemistry on a Perkin-Elmer 391 synthesizer. Unrelated Y2 DNA oligonucleotide was obtained in the same manner. All reagents and liquid handling material coming into contact with the RNA were either certified RNAse-free or treated with diethylpyrocarbonate (J. Sambrook and D. W. Russel, Molecular Cloning, A Laboratory Manual, CSHL Press, 2001) to inactivate RNAses. Fluorescence measurements were performed at 42.5°C on a Variant Cary Eclipse spectrofluorometer with excitation set at 420±10 nm and fluorescence emission measured at 530±5 nm, applying 1000 volts to the detector.

A duplex between polymer 2 and oligo N1 was formed by contacting  $8.66 \times 10^{13}$  copies of oligo N1 with the equivalent amount of positive charges of polymer 2. The reaction was carried out at room temperature for thirty seconds in 2 µL of water. One µl of the duplex

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mixture was diluted in 2 mL of water and put into a quartz cuvette for measurement of the initial fluorescence signal. Thereafter, a triplex was formed at 42.5°C by adding and mixing  $8.66 \times 10^{13}$  copies of heat treated (2 minutes at 95°C) NAC1 messenger RNA. Fluorescence of the triplex  
5 was measured. A similar duplex and triplex was also made using the DNA oligonucleotide Y2, which has no significant homology with the sequence of the NAC1 messenger RNA.

The fluorescence was significantly higher for the N1/polymer 2/NAC1 triplex than for the Y2/polymer 2/NAC1 triplex, thereby  
10 showing the ability of polymer 2 to distinguish between specific and nonspecific hybridization of 20 mers DNA oligonucleotides with messenger RNA.

In conclusion, a novel methodology has been developed  
15 that allows the detection of nucleic acids by simple optical and electrochemical means. This rapid, selective, and versatile method does not require any chemical reaction on the probes or the analytes, and is based on different electrostatic interactions and conformational structural changes between cationic poly(3-alkoxy-4-methylthiophene) derivatives  
20 and single-stranded oligonucleotides or double-stranded (hybridized) nucleic acid fragments. The present polymer-based technology is simple and specific and provides a flexible platform for the rapid detection of nucleic acids.

The polythiophenes are thermostable and autoclavable,  
25 hence allowing for a wide range of applications.

The terms and descriptions used herein are preferred embodiments set forth by way of illustration only, and are not intended as limitations on the many variations which those of skill in the art will

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recognize to be possible in practicing the present invention, as defined in the following claims.

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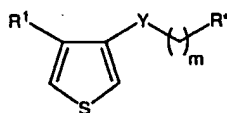
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**Claims**

1. A thiophene monomer having the following general formula:



wherein:

- 5           a) "m" is an integer ranging from 2 to 3;  
              b) R\* is a quaternary ammonium;  
              c) Y is an oxygen atom or a methylene; and  
              d) R¹ is a methyl group or a hydrogen atom.

10                       2. A monomer as defined in claim 1, wherein:

- a) "m" = 3;  
      b) R\* is <sup>+</sup>NEt<sub>3</sub>;  
      c) Y is an oxygen atom; and  
      d) R¹ is a methyl group.

15

3. A monomer as defined in claim 1, wherein:

- a) "m" is = 2;  
      b) R\* is



20

- c) Y is an oxygen atom; and  
      d) R¹ is a methyl group.

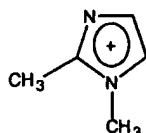
25

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4. A monomer as defined in claim 1, wherein:

a) "m" is = 2;

b) R\* is



5

c) Y is an oxygen atom; and

d) R<sup>1</sup> is a methyl group.

5. A monomer as defined in claim 1, wherein:

a) "m" is = 2;

b) R\* is



15

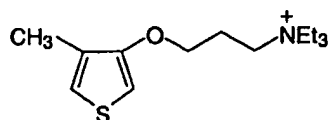
c) Y is a methylene group; and

d) R<sup>1</sup> is a hydrogen atom.

6. The monomer as defined in claim 2, having the

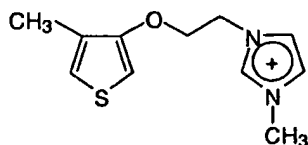
formula:

20



7. The monomer as defined in claim 3, having the

25 formula:

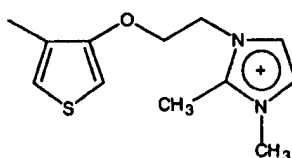




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8. The monomer as defined in claim 4, having the

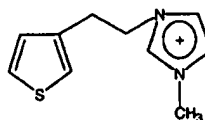
formula:



5

9. The monomer as defined in claim 5, having the

formula:



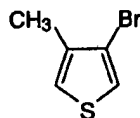
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10. A polymer comprising a plurality of monomeric units as defined in any one of claims 1 to 9.

15

11. A process for the preparation of a monomer as defined in claim 2, comprising:

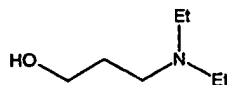
a) reacting a molecule of formula 1:



20

formula 1

with a reagent of formula 2:



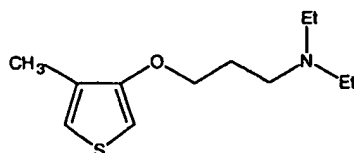
25

formula 2

- 40 -

in the presence of a copper halide, said reaction resulting in the formation of a first reaction mixture comprising a compound of formula 3:

5



formula 3

b) isolating said compound of formula 3 from said first reaction mixture;

c) reacting said compound of formula 3 with ethylbromide, resulting in the formation of a second reaction mixture comprising said monomer; and

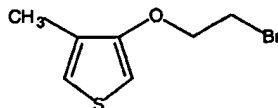
d) recovering said monomer from said second reaction mixture.

15

12. The process of claim 11, wherein said copper halide is CuI.

13. A process for the preparation of a monomer as defined in claim 3, comprising:

20 a) reacting a compound of formula 4:



formula 4

25

with 1-methylimidazole, resulting in the formation of a reaction mixture; and  
b) recovering said monomer from said reaction mixture.

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14. A process for the preparation of a monomer as defined in claim 4, comprising:

a) reacting a compound of formula 4:



formula 4

with 1,2-dimethylimidazole, resulting in the formation of a reaction mixture; and

10 b) recovering said monomer from said reaction mixture.

15. A process for the preparation of a monomer as defined in claim 5, comprising:

a) reacting a compound of formula 6:



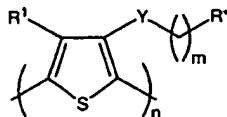
formula 6

with 1-methylimidazole, resulting in the formation of a reaction mixture; and

20 b) recovering said monomer from said reaction mixture.

16. A process for polymerizing the monomer defined in any one of claims 1 to 9, comprising reacting said monomer in the presence of an oxidizing agent, resulting in the formation of a polymer of the following general formula:

25



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wherein:

- 5           a) "m" is an integer ranging for 2 to 3;  
            b) "n" is an integer ranging from 3 to 100;  
            c) R\* is a quaternary ammonium;  
            d) Y is an oxygen atom or a methylene; and  
            e) R<sup>1</sup> is a methyl group or a hydrogen atom.

10

17. The process of claim 16, wherein said oxidizing agent is FeCl<sub>3</sub> or K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>.

15           18. The process of claim 16, wherein when R<sup>1</sup> is a methyl group, said polymer is regio-regular and water soluble.

19. The process of claim 18, wherein said polymer is thermostable.

20           20. A polymer obtained from the process of anyone of claims 16 to 18.

21. A method for detecting the presence of a negatively charged polymer, comprising the steps of:

- 25           a) contacting a complementary target to said polymer with a polymer as defined in claim 20 to form a duplex;  
            b) contacting said duplex with said negatively charged polymer; and

- 43 -

c) detecting a change in electronic charge, fluorescence or color as an indication of the presence of said negatively charged polymer.

22. A method as defined in claim 21, wherein said  
5 negatively charged polymer is selected from the group consisting of: acidic proteins, glycosaminoglycans, hyaluronans, heparin, chromatographic substrates, culture substrates and nucleic acids.

23. A method as defined in claim 22, wherein said  
10 negatively charged polymer is a nucleic acid.

24. A method as defined in claim 23, wherein said nucleic acid is DNA.

15 25. A method as defined in claim 23, wherein said nucleic acid is RNA.

26. A method as defined in claim 23, wherein said  
complementary target is a nucleic acid complementary to said negatively  
20 charged polymer.

27. A method as defined in claim 26, wherein said  
complementary target is a nucleic acid probe and said negatively charged  
polymer is a denatured PCR amplicon.

25

28. A method for discriminating a first nucleic acid from a second nucleic acid, said second nucleic acid differing from said first nucleic acid by at least one nucleotide, comprising the steps of:

- 44 -

- e) contacting a complementary target to said first nucleic acid with a polymer as defined in claim 20 to form a duplex;
- f) contacting said duplex with said first nucleic acid, resulting in specific hybridization;
- 5 g) contacting said duplex with said second nucleic acid, resulting in non-specific hybridization; and
- h) detecting a change in electronic charge, fluorescence or color.

29. A method as defined in claim 28, wherein said  
10 change in electronic charge, fluorescence or color is more pronounced with said specific hybridization.

30. A method as defined in claim 29, wherein said  
15 complementary target is a nucleic acid complementary to said first nucleic acid.

31. A method as defined in claim 30, wherein said first nucleic acid and said second nucleic acid are DNA.

20 32. A method as defined in claim 30, wherein said first nucleic acid and said second nucleic acid are RNA.

33. A method as defined in claim 30, wherein said  
25 complementary target is a nucleic acid probe and said first nucleic acid and said second nucleic acid are denatured PCR amplicons.

34. Use of a positively charged polymer comprising a repeating thiophene moiety for detecting the presence of a negatively charged polymer.

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35. A use as defined in claim 34, wherein said positively charged polymer is reacted with a complementary target to said negatively charged polymer.

5

36. A use as defined in claim 35, wherein said positively charged polymer is ionically linked to said complementary target.

37. A use as defined in claim 34, wherein a  
10 complementary target to said negatively charged polymer is linked to a solid support, said negatively charged polymer is then reacted with said complementary target which results in its capture by said solid support resulting in the formation of a complex and then said positively charged polymer is reacted with said complex of said complementary target with  
15 said negatively charged polymer.

38. A use as defined in claim 34, wherein a  
complementary target to said negatively charged polymer is linked to a solid support, said positively charged polymer is then reacted with said  
20 complementary target resulting in its capture by said solid support forming a complex, and then reacting said negatively charged polymer with said complex of said complementary target and said positively charged polymer.

25 39. A use as defined in claim 37 or 38, wherein said solid support is selected from the group consisting of an electrode, an optical fiber, a glass slide and glass beads.

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40. A use as defined in any one of claims 37-39 for purifying negatively charged polymers.

41. A use as defined in claim 40, wherein said  
5 negatively charged polymers are nucleic acids.



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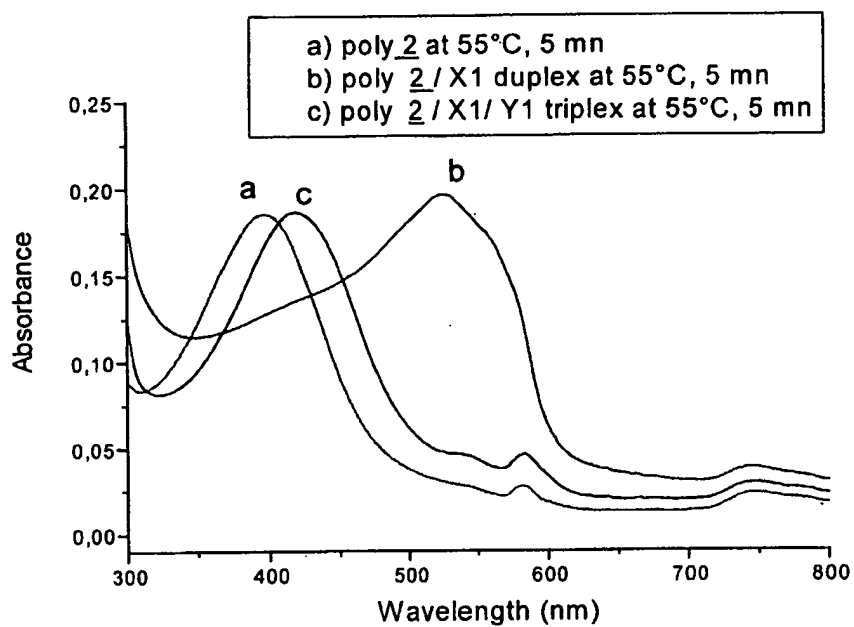


Figure 1

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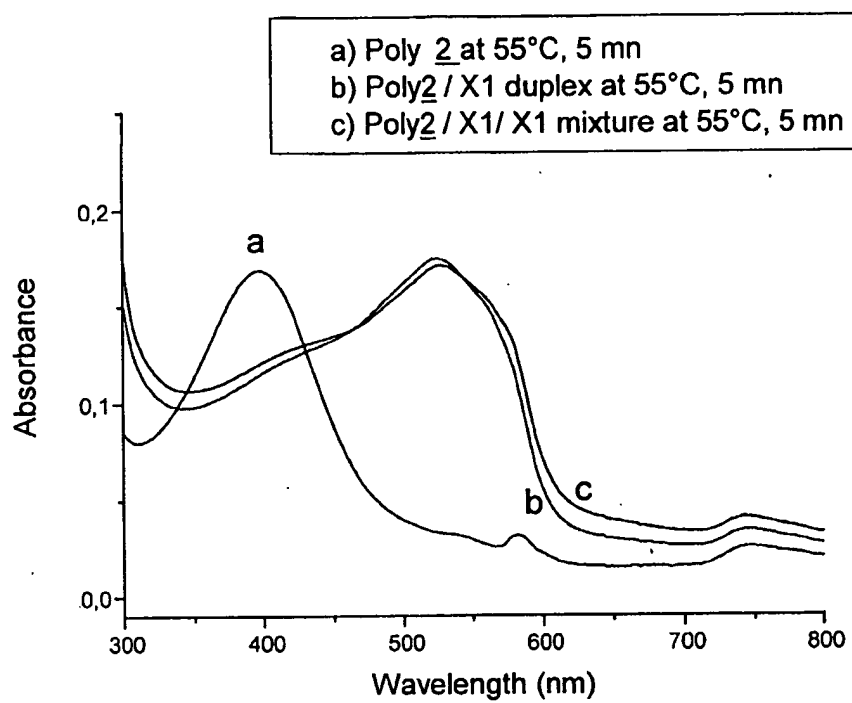


Figure 2

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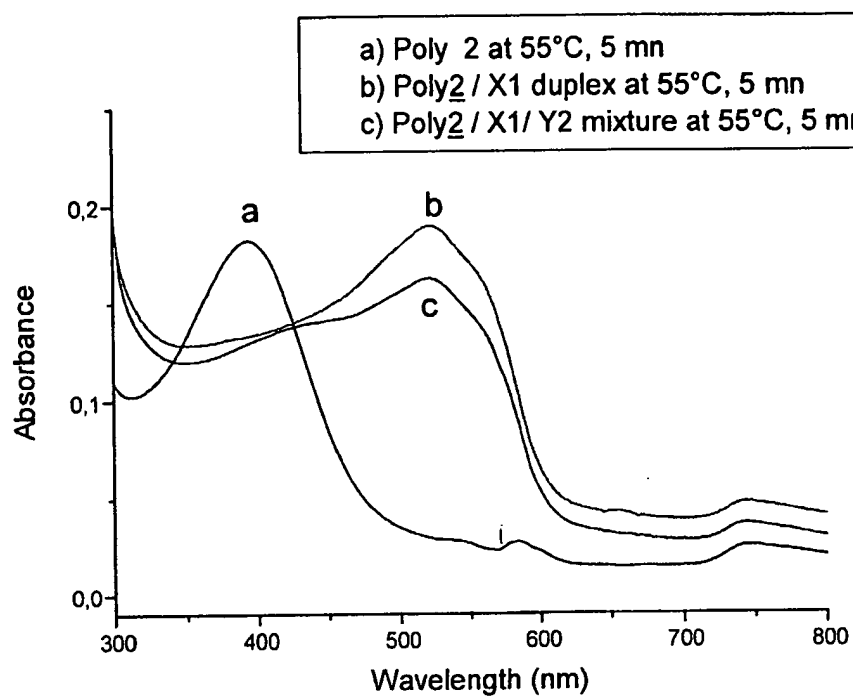


Figure 3

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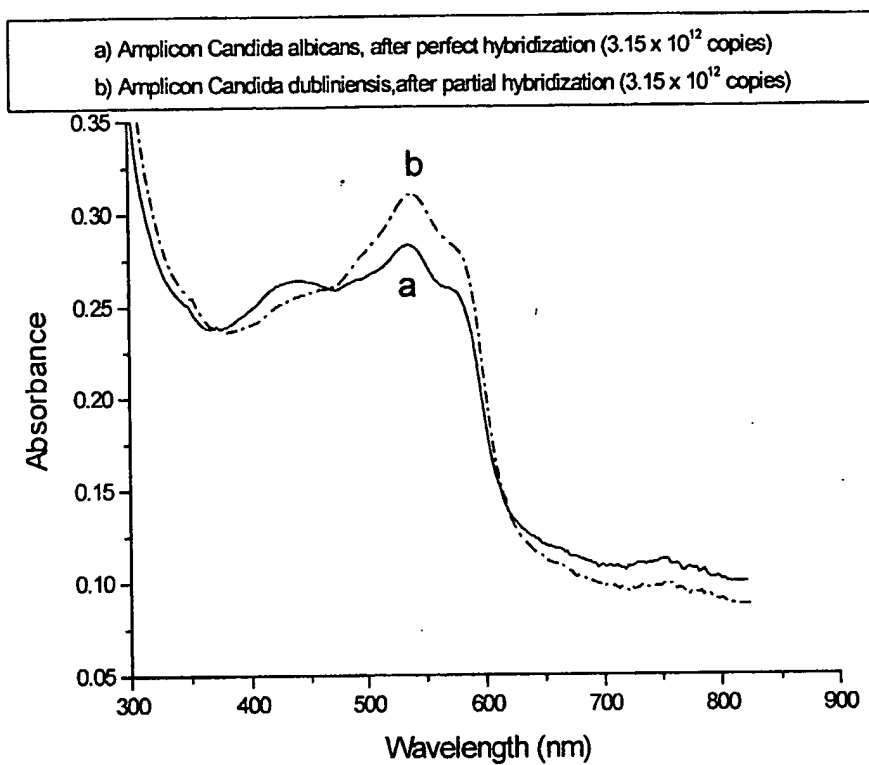


Figure 4

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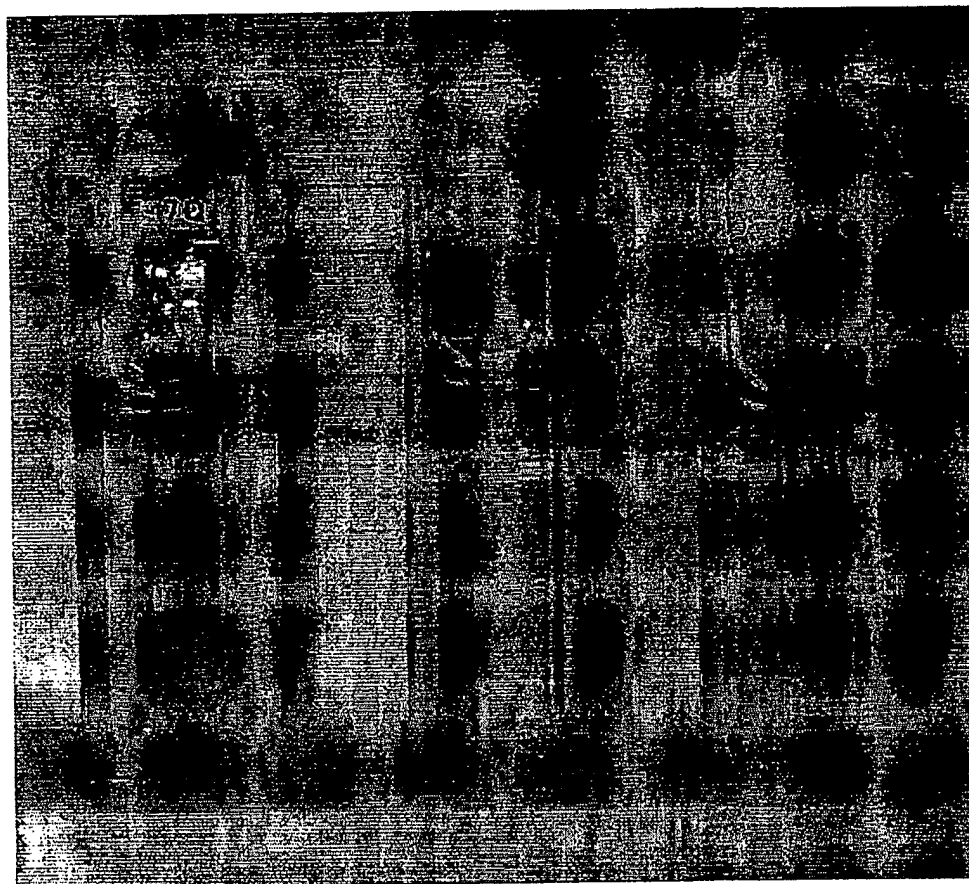


Figure 5

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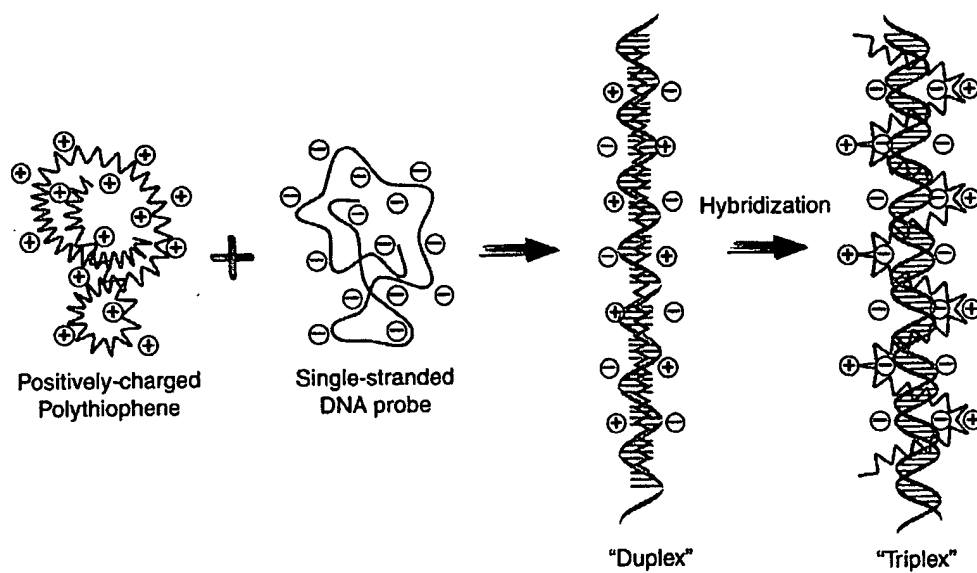


Figure 6

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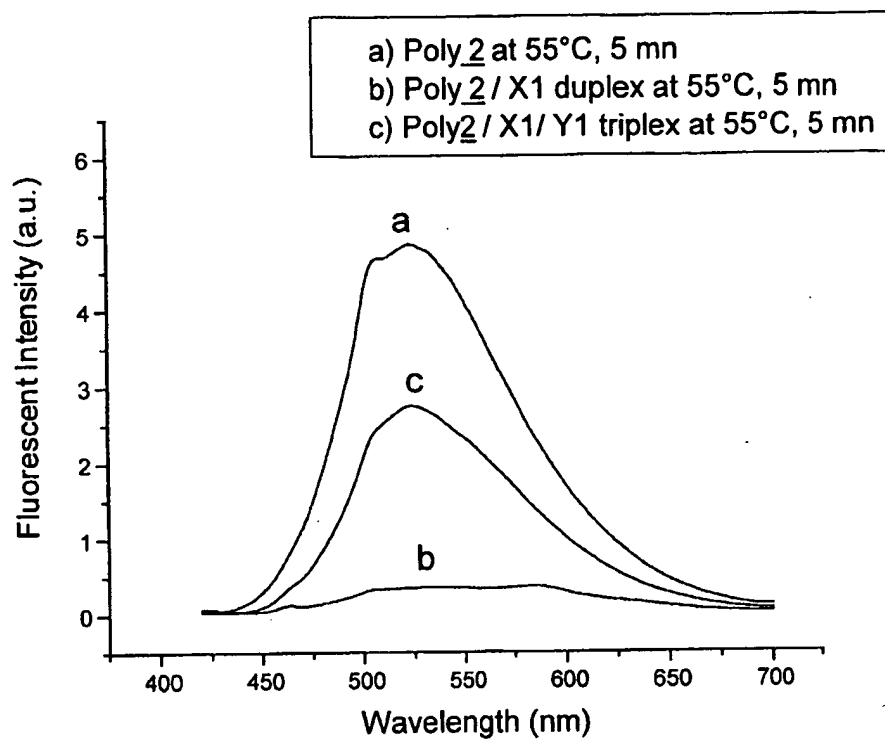


Figure 7

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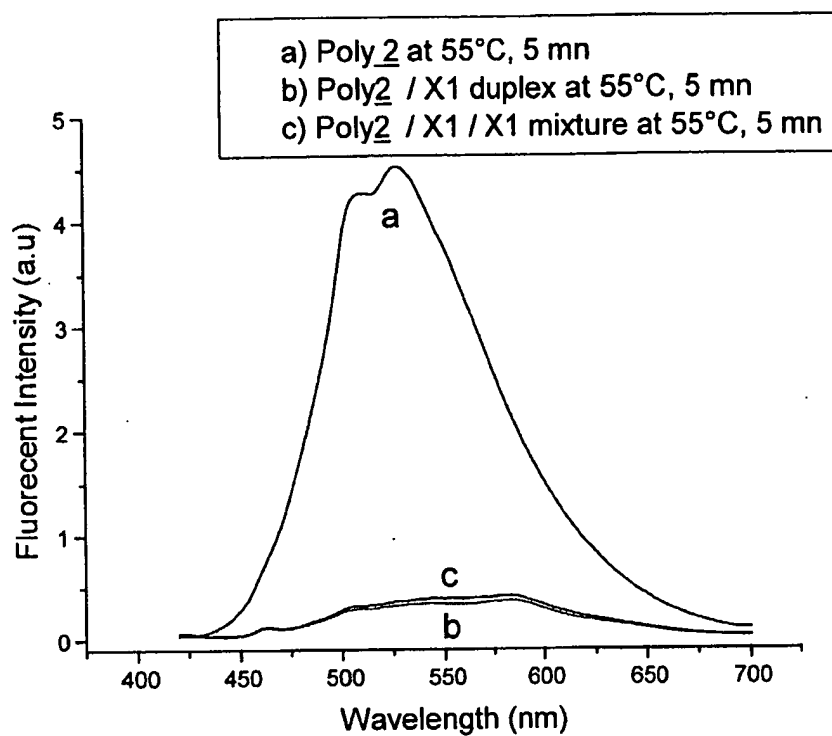


Figure 8



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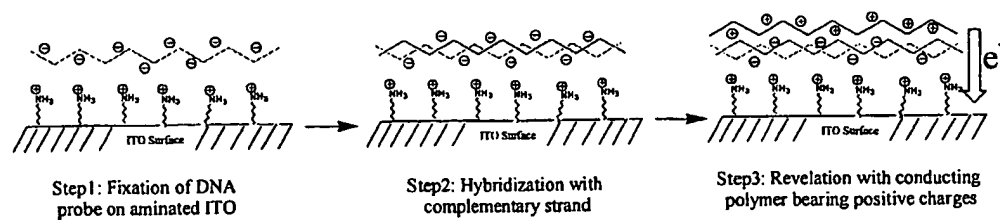


Figure 9

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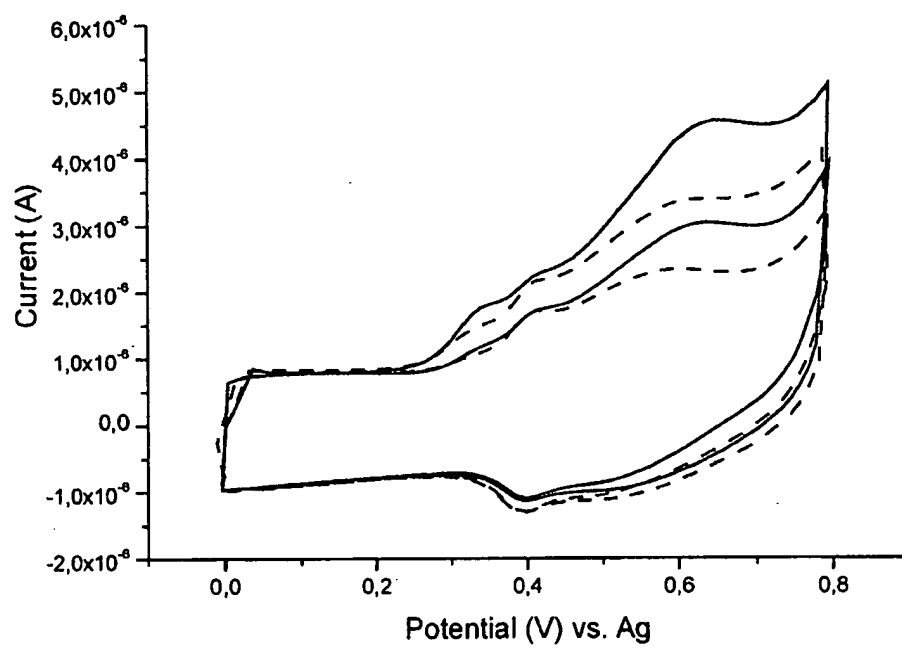


Figure 10

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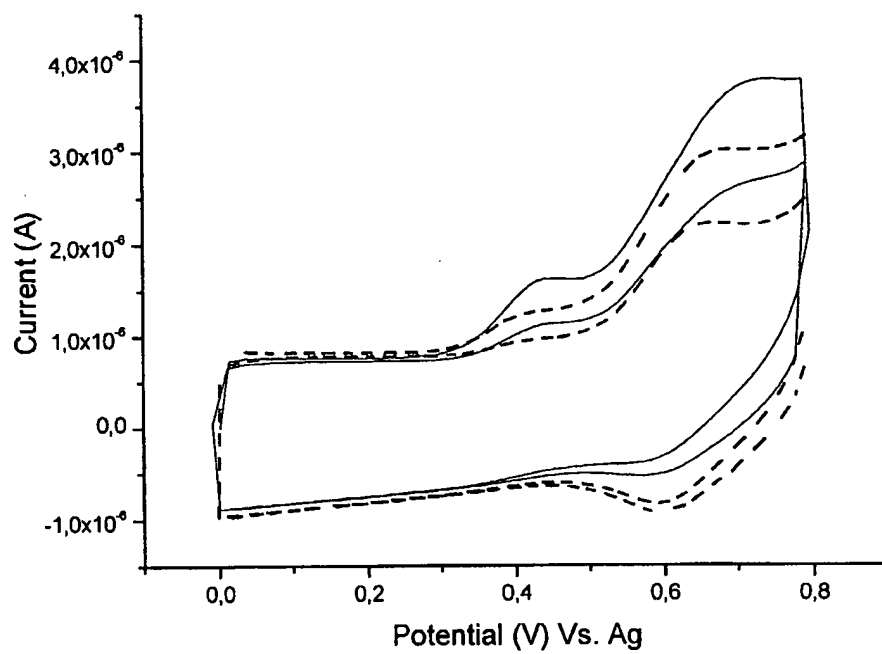


Figure 11

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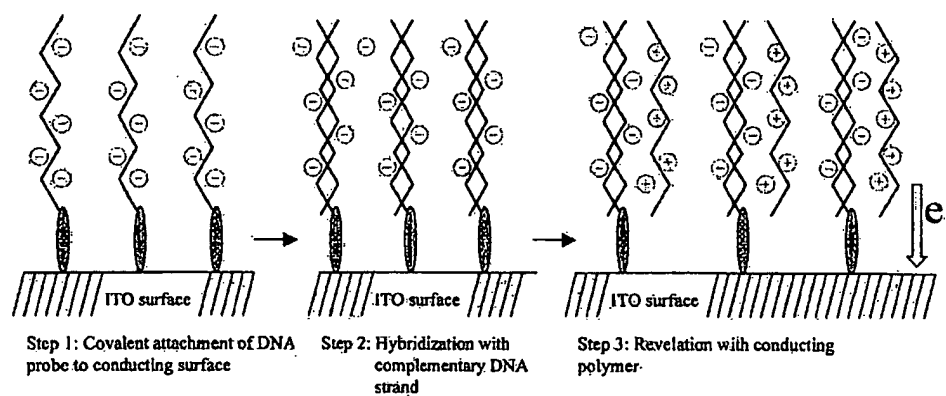


Figure 12

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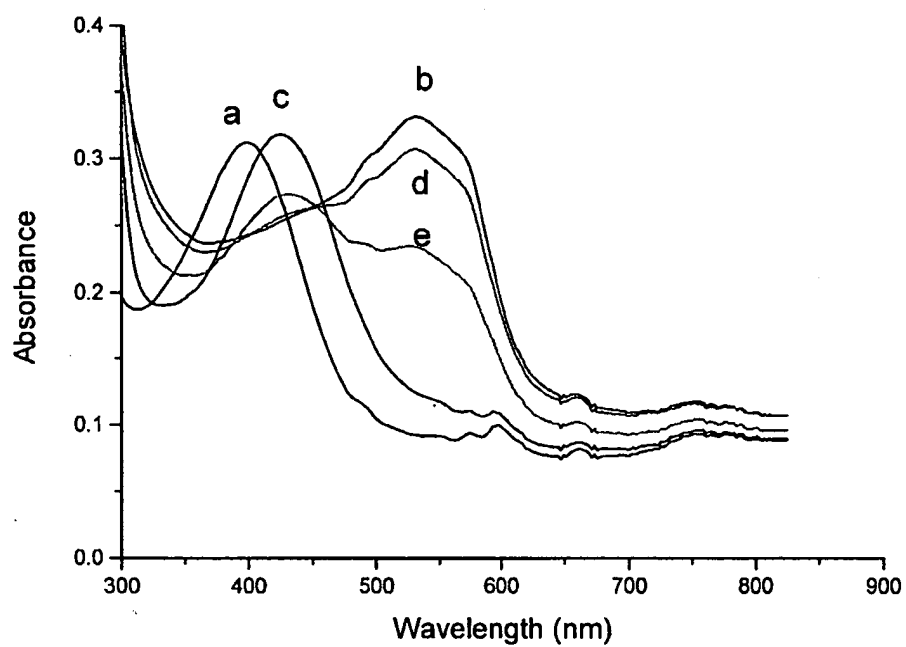


Figure 13

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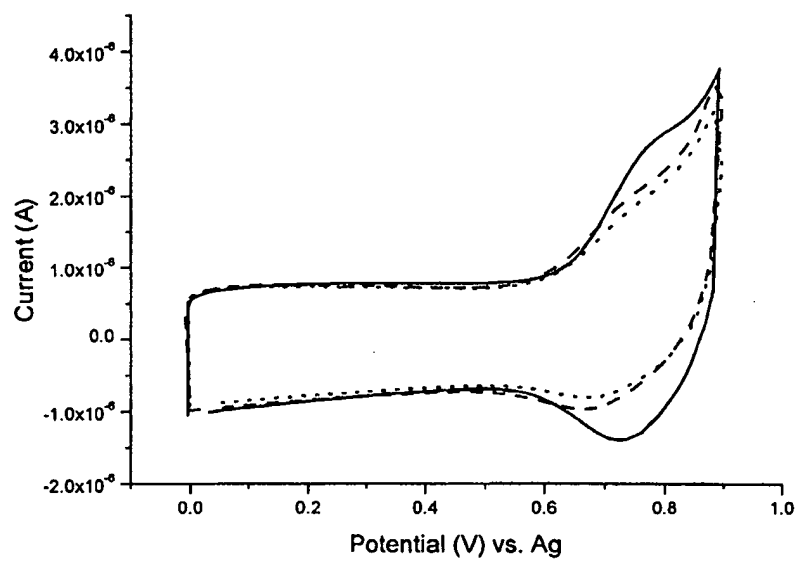


Figure 14

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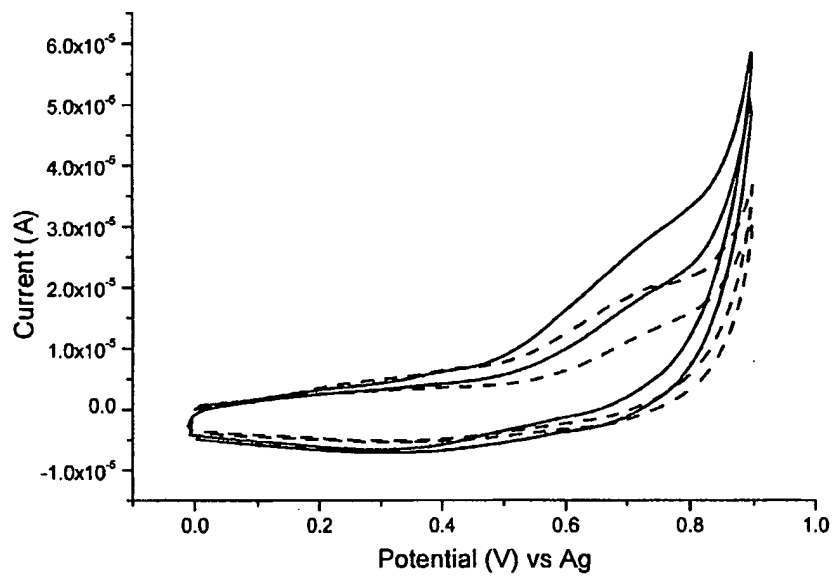


Figure 15

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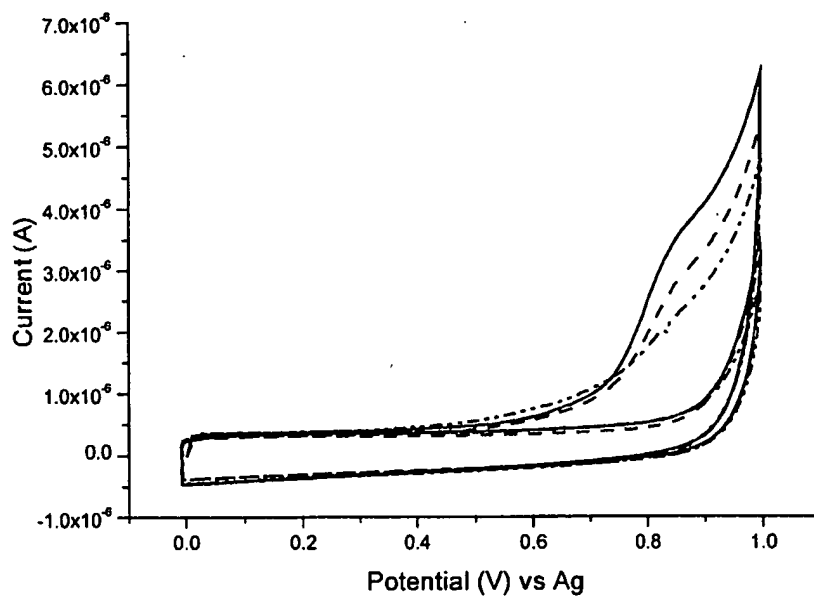


Figure 16



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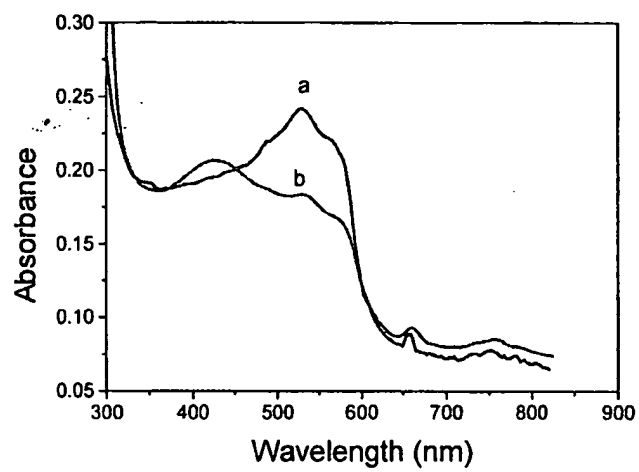


Figure 17

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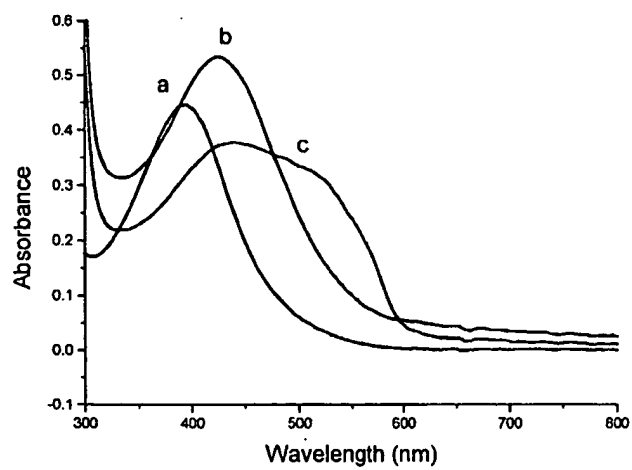


Figure 18

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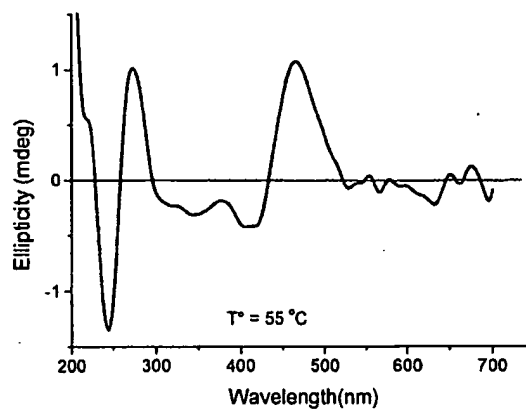


Figure 19

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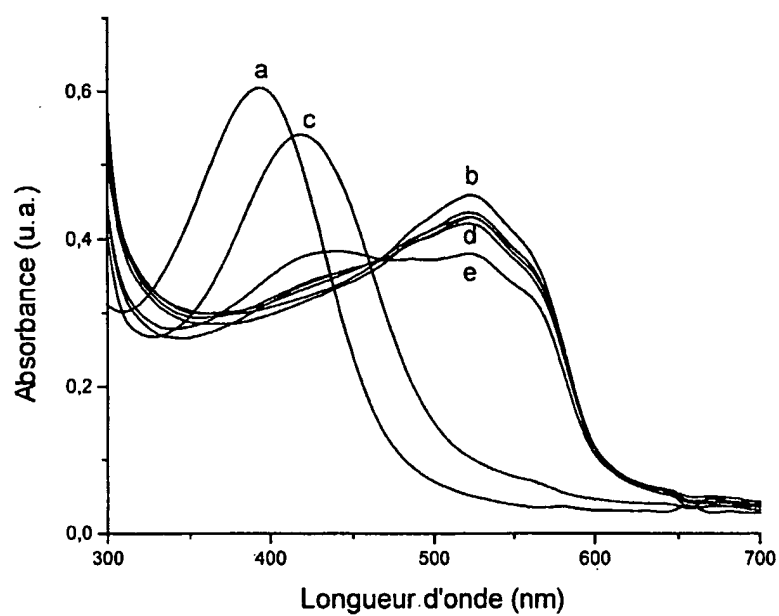


Figure 20

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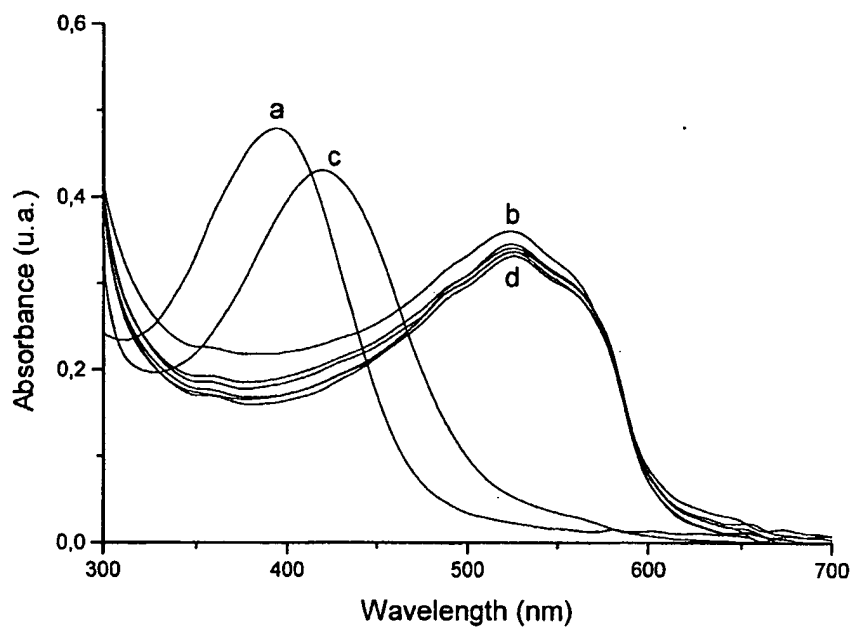


Figure 21